Note

Food Composition and Emulsifying Activity of Lipoxygenase Deficient Mutant Soybeans

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The consumption of soy proteins as food ingredients is being encouraged, since soy protein is not only an economical food resource, but it is also high in the qualities of nutrition and functionality.1,2 However, its characteristic grassy beany and green flavors build a barrier against the wide utilization of soy protein. Major contributors to these objectionable flavors are the volatile carbonyl compounds, which are enzymatically derived from hydroperoxides of unsaturated fatty acids.3 Soybean contains three kinds of lipoxygenase isozymes (L-1, L-2 and L-3) which exhibit different kinetic behaviors.4,5 The following mechanism for the formation of n-hexanal, one of the major elements of soybean flavors, has been proposed in soybean homogenate: The L-2 isozyme predominantly reacts with linoleic acid to produce 13-hydroperoxide of linoleic acid under the usual conditions (pH 6~7), and the hydroperoxide is cleaved by hydroperoxide lyase to produce n-hexanal.7 Furthermore, the rate-determining step of n-hexanal formation from linoleic acid is the step of the 13-hydroperoxide formation by lipoxygenase.7 The evidence suggests that a key enzyme for n-hexanal formation under the processing and storage of soybeans is lipoxygenase(s). Therefore, soybeans lacking lipoxygenase isozymes are expected to be useful for preparing soy proteins with a low level of grassy beany and green flavors.

Recently, soybean seeds that lacked lipoxygenase isozymes were found as the results of screening a variety of seeds8-10. In the present study, in order to elucidate whether such lipoxygenase deficient mutant seeds are useful as food resources, we show some food characteristics of the mutant seeds (L-1 null, L-2 null, L-3 null and L-1, 3 null) in comparison with those of normal seed, with respect to the food composition of the seeds, the amino acid composition, the content of 7S and 11S globulins and the emulsifying activity of proteins from the seeds.

Soybeans were grown at the Iwate University Experimental Farm located in Morioka, Iwate in 1984. Normal seed was Glycine max var. Suzuyutaka. The L-1 deficient mutant was a line from the cross between P.I. 408251 (L-1 null type) and Suzuyutaka. The L-2 deficient mutant was a line from the cross between P.I. 86023 (L-2 null type) and Suzuyutaka. The L-3 deficient mutant was a line from the cross between Tohoku No. 74 (L-3 null type) and Suzuyutaka. The L-1 and L-3 double mutant was a line from the cross between Wasenatsu (L-3 null type) and P.I. 408251.

Soy protein was prepared as follows: Soybean seeds (10 grains, about 2 g) were soaked overnight in distilled water at 4°C. After removal of the seed coat, the soaked seeds (about 3 g) were homogenized in 30 ml of 33 mM phosphate buffer (pH 7.6) containing 0.4 mM 2-mercaptoethanol and 0.4 mM NaCl with a Waring blender at 0°C. The homogenate was centrifuged for 30 min at 8,000 rpm. Chilled acetone (30 ml) was slowly added to 20 ml of the supernatant at 0°C under stirring. The precipitate obtained was collected by centrifugation for 10 min once and followed by washing with chilled ethyl ether. The resulting powder was dried in a desiccator over phosphorous pentoxide in vacuo. The powder (300 mg) dissolved in 30 ml of the buffer was dialyzed against the buffer overnight. After removal of insoluble materials, the pH of the clear solution was adjusted to pH 4.6 with 1 N HCl. The resulting precipitate was dialyzed against the buffer overnight. The clear solution obtained by centrifugation was used for the following experiments.

The protein content was determined by the procedure of Lowry et al. with bovine serum albumin as the standard.11 Quantitative amino acid determination was carried out with an amino acid analyzer (Hitachi 835). The protein was hydrolyzed with re-distilled 6 N HCl at 110°C for 24 hr in an evacuated tube in the presence of 1% phenol. Tryptophan and cysteine were not determined.

The emulsifying activity was determined by the turbidimetric method of Pearch and Kinsella.12 Soybean oil was added to 1.5 ml of 0.4% protein solution in 33 mM phosphate buffer (pH 7.6) containing 0.4 mM NaCl and 10 mM 2-mercaptoethanol. The amount of soybean oil was varied in the range of 0.1, 0.3, 0.5 and 1.0 ml. The mixture was emulsified for 1 min by sonication. Ten µl of the emulsion was mixed with 10 ml of 1% sodium dodecyl sulfate and the absorbance at 600 nm was measured. The data were expressed as relative values (percentage) compared with the protein solution for normal seed (Suzuyutaka).

Analytical linear sucrose density gradient centrifugation was performed as follows. Five mg of protein in 0.4 ml of 33 mM phosphate buffer (pH 7.6) containing 0.4 mM NaCl and 10 mM 2-mercaptoethanol was layered on top of 15 ml...
of linear sucrose density gradient (10–30%) in the sample buffer. The whole was centrifuged with a Hitachi RPS 40T rotor at 36,000 rpm and at 20°C for 16 hr. After centrifugation, the gradient was divided into 0.4 ml fraction with an ISCO density gradient fractionator.

The food composition (moisture, crude protein, crude fat and carbohydrate) was measured by the near infrared spectroscopy method. This measurement was carried out with a near infrared spectrometer (Neotec 4250), using the ground soybean sample (10 g).

The food composition of soybeans (L-1 null, L-2 null, L-3 null and L-1, -3 null) lacking lipoxygenase isozymes and of normal seed (Suzuyutaka) was measured. The composition of each component varied little among all the seeds. The amino acid composition of protein from the soybeans lacking lipoxygenase isozymes and the normal seed was also measured, indicating that the composition was very similar among all the seeds. This data was insignificantly different from that reported by Catsimpoolas et al. 13)

Lipid analysis of all the soybeans has already been described. 6) In all the seeds, the amounts of triacylglycerol, phospholipid, diacylglycerol and free fatty acid were 93–96%, 3–6%, 0.6–0.9% and 0.1–0.3%, respectively. The content of linoleic acid was the highest and that of stearic acid was the lowest. The fatty acid composition of each lipid class was similar among all the seeds. These results were insignificantly different from those of normal types of soybeans. 14)

The proportions of the components of storage proteins from soybeans lacking lipoxygenase isozymes were determined. The centrifugal profiles are shown in Fig. 1, indicating that the storage proteins of lipoxygenase deficient soybeans (Fig. 1, B ~ E) consisted of 2S, 7S, 11S and 15S globulins, which are the components of storage proteins in the normal type of soybeans. The content of 11S globulin was higher in L-3 null and L-1, -3 null seeds than in the other seeds. The total content of 7S and 11S globulins was found to be similar among all the seeds as a result of calculation from the peak area of the centrifugal profiles. Saio et al. reported that proportions of 7S and 11S globulins are different among several cultivars of soybeans. 15)

The emulsifying activity of proteins from all the seeds was measured as shown in Table I. The activity was the highest in L-1 null seed and the lowest in L-1, -3 null seed. The difference of activity may be due to the ratio of 7S and 11S globulins, since Aoki et al. reported that the emulsifying capacity and stability of 7S globulin are higher than those of 11S globulin. 16)

From the several lines of evidence, it is likely that lipoxygenase deficient mutant soybeans are useful as food resources, since the composition of mutant soybeans is very similar that of the normal type of soybeans.

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